

Figure 1

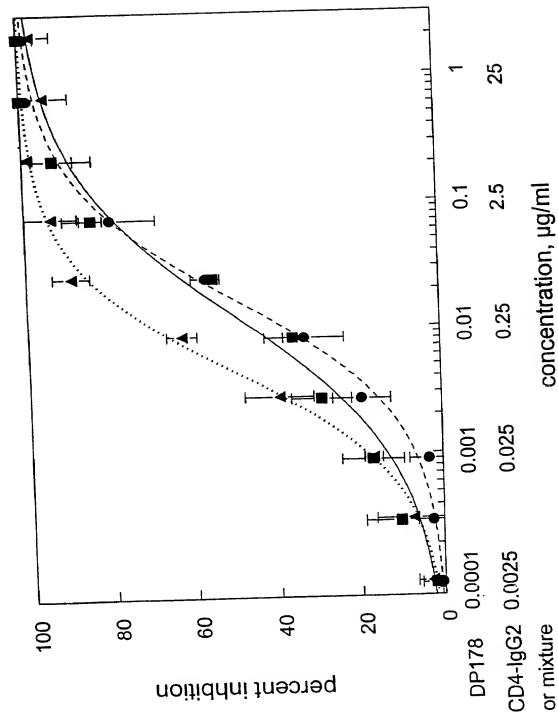


Figure 2

Percent Inhibition	Combination Index			
	CD4-IgG2:T-20 Mass Ratio			
	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 3

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, $\mu\text{g/ml}$		Dose Reduction	Concentration, $\mu\text{g/ml}$		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 4A

Percent Inhibition	Combination Index	PRO 542			PA12			T-20		
		Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
		Alone	Mix		Alone	Mix		Alone	Mix	
95	0.41	10	2.1	4.8	730	2.8	260	94	19	4.9
90	0.45	7.0	1.6	4.4	320	2.1	150	63	14	4.5
70	0.47	4.1	0.92	4.5	72	1.2	60	30	8.1	3.7
50	0.48	3.1	0.66	4.7	28	0.87	32	19	5.8	3.3

PRO 542, PA12 and T-20 were used in an approximate 1:1:10 molar concentration ratio.

Figure 4B

Percent Inhibition	Combination Index	PRO 542			PRO 140			T-20		
		Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
		Alone	Mix		Alone	Mix		Alone	Mix	
95	0.40	8.5	1.9	4.5	19	1.0	19	140	17	8.2
90	0.39	7.1	1.5	4.7	13	0.77	17	100	13	7.7
70	0.37	5.3	0.87	6.1	7.2	0.46	16	57	7.7	7.4
50	0.35	4.6	0.63	7.3	4.9	0.34	14	40	5.6	7.1

PRO 542, PRO 140 and T-20 were used in an approximate 2:1:20 molar concentration ratio.

Figure 4C

Percent Inhibition	Combination Index	PRO 542			PRO 140			T-20		
		Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
		Alone	Mix		Alone	Mix		Alone	Mix	
95	0.24	61	2.5	24	11.9	0.72	17	156	22	7.1
90	0.22	32	1.4	23	8.4	0.40	21	96	13	7.4
70	0.19	9.8	0.50	20	4.5	0.14	32	40	4.5	8.9
50	0.18	4.7	0.26	18	3.0	0.074	41	23	2.3	10

PRO 542, PRO 140 and T-20 were used in an approximate 4:1:30 molar concentration ratio.

Figure 4D

Percent Inhibition	Combination Index	PRO 140		T-20	
		Concentration, nM		Concentration, nM	
		Alone	Mix	Alone	Mix
95	0.56	12	1.8	6.7	156
90	0.55	8.4	1.1	7.4	96
70	0.55	4.5	0.51	8.8	40
50	0.56	3.0	0.31	9.9	23

PRO 140 and T-20 were used in an approximate 1:30 molar concentration ratio.

Triple Combination Synergistically Blocks HIV-1 Entry (1)

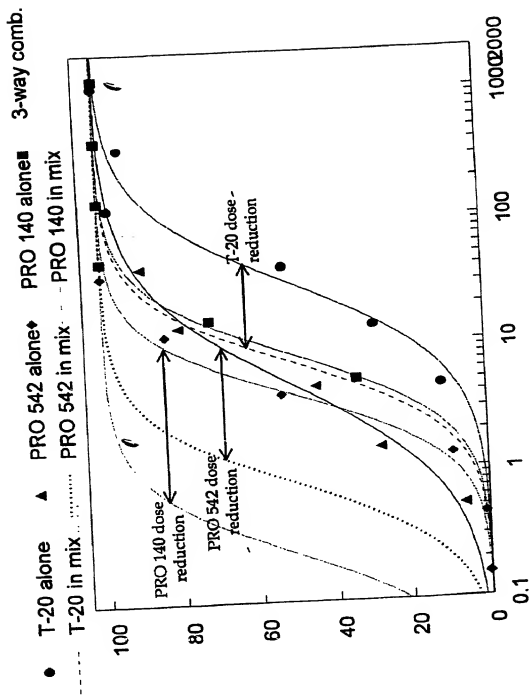


Figure 6

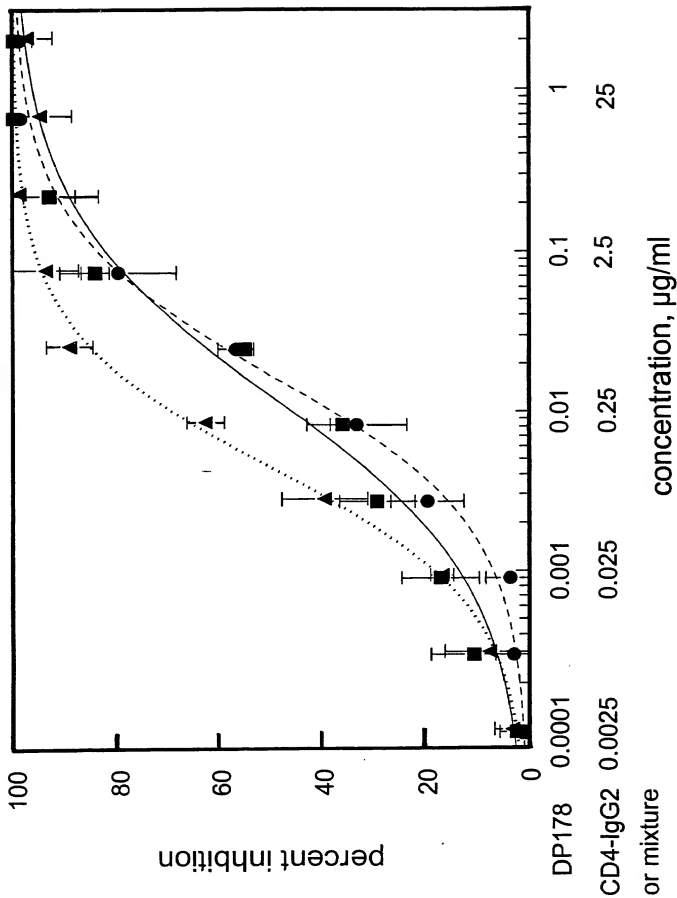


Figure 7

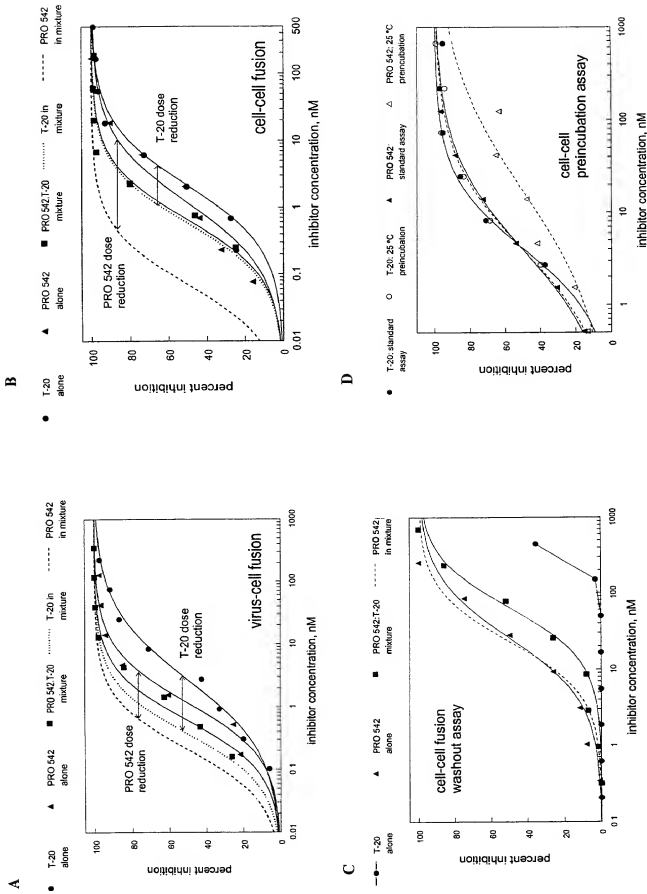
Combination Index				
CD4-IgG2:T-20 Mass Ratio				
Percent Inhibition	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 8

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, $\mu\text{g/ml}$		Dose Reduction	Concentration, $\mu\text{g/ml}$		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Assay (virus)	PRO 542:T-20 Molar Ratio	Percent Inhibition	Combination Index	PRO 542			T-20		
				Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
				Alone	Mix		Alone	Mix	
Virus-cell fusion (JR-FL)	1:2	95	0.14	30	2.8	11	120	5.1	24
		90	0.18	12	1.5	8.0	45	2.6	17
		70	0.29	2.5	0.44	5.7	8.0	0.78	10
		50	0.39	0.92	0.21	4.4	2.7	0.37	7.3
Virus-cell fusion (DH'23)	1:2	95	0.36	65	11	5.9	123	20	6.2
		90	0.45	20	5.0	4.0	54	8.9	6.1
		70	0.76	2.4	1.2	2.0	12	2.1	5.7
		50	1.1	0.64	0.49	1.3	4.8	0.87	5.5
Cell-cell fusion (JR-FL)	1:2	95	0.36	35	6.3	5.6	73	11	6.6
		90	0.43	14	3.2	4.4	34	5.8	5.9
		70	0.61	2.9	0.94	3.1	8.5	1.7	5.0
		50	0.76	1.0	0.43	2.3	3.6	0.78	4.6
Cell-cell fusion (JR-FL)	1:10	95	0.27	28	1.4	20	58	12	4.8
		90	0.28	11	0.55	20	22	4.9	4.5
		70	0.31	2.3	0.11	21	3.8	0.97	3.9
		50	0.34	0.84	0.039	17	1.3	0.35	3.7
Cell-cell fusion (JR-FL)	1:50	95	0.33	47	0.84	56	120	37	3.2
		90	0.34	15	0.30	50	42	13	3.2
		70	0.36	1.8	0.045	40	6.1	2.0	3.0
		50	0.38	0.49	0.014	35	1.8	0.61	3.0

Figure 10



HIV-1 Entry Involves at Least Three Steps that Provide Promising Targets for Therapy

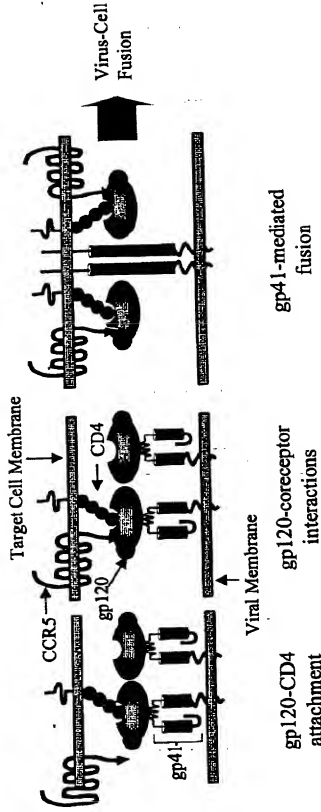
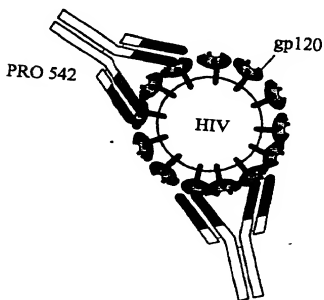


Figure 12

PRO 542 (CD4-IgG2)
attachment inhibitor



PRO 140
coreceptor inhibitor

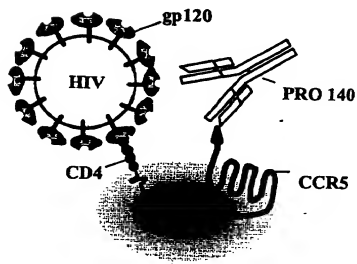
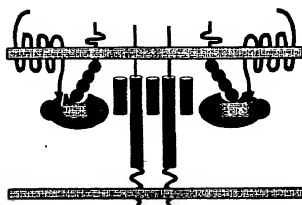


Figure 14

T-20
fusion inhibitor



HIV-1 Virus-Cell Fusion Assay

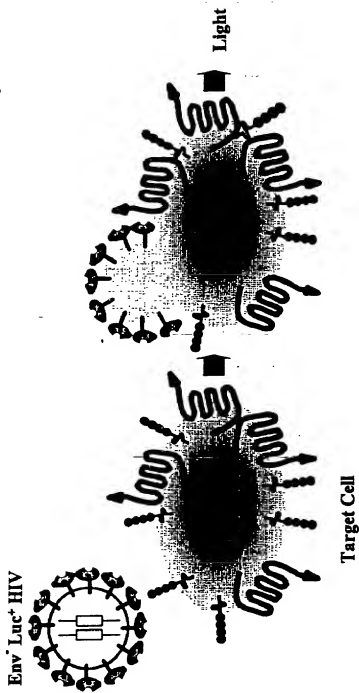
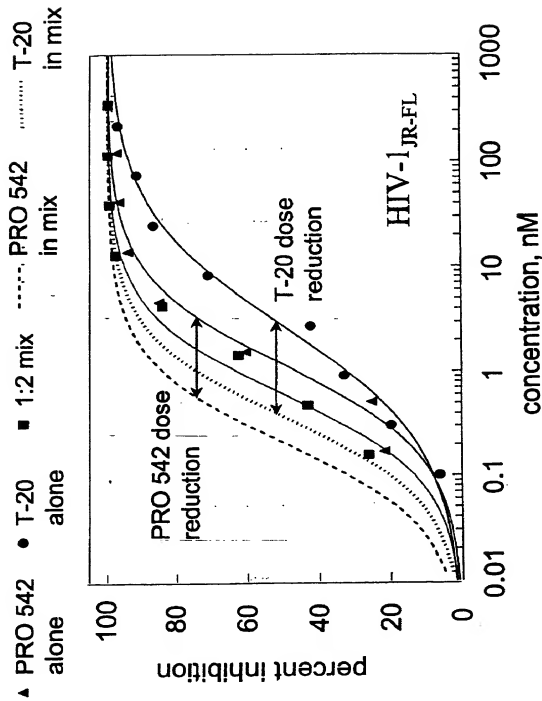


Figure 16

Synergistic Inhibition of Virus-Cell Fusion with PRO 542 and T-20 (I)



Synergistic Inhibition of HIV-1 Virus-Cell Fusion with PRO 542 and T-20 (II)

Percent Inhibition	Combination Index	Inhibitory Conc., nM		Dose Reduction	
		PRO 542	T-20	PRO 542	T-20
JR-FL 95	0.14	30	120	11	24
(R5) 90	0.18	12	45	8.0	17
70	0.29	2.5	8.0	5.7	10
50	0.39	0.92	2.7	4.4	7.3
DH123 95	0.36	65	123	5.9	6.2
(R5X4) 90	0.45	20	54	4.0	6.1
70	0.76	2.4	12	2.0	5.7
50	1.1	0.64	4.8	1.3	5.5

PRO 542 and T-20 were used in a 1:2 molar ratio

Figure 18

HIV-1 Cell-Cell Fusion Assay

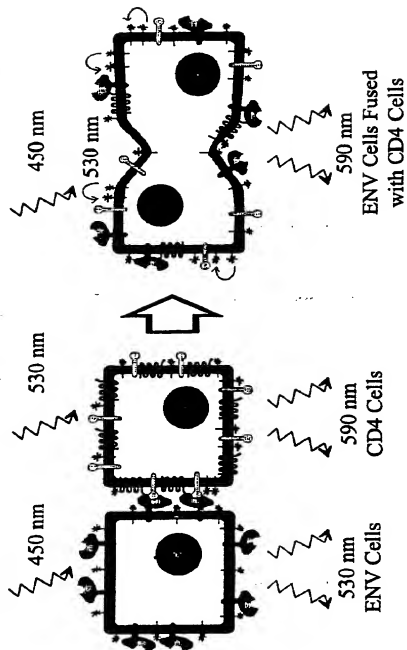
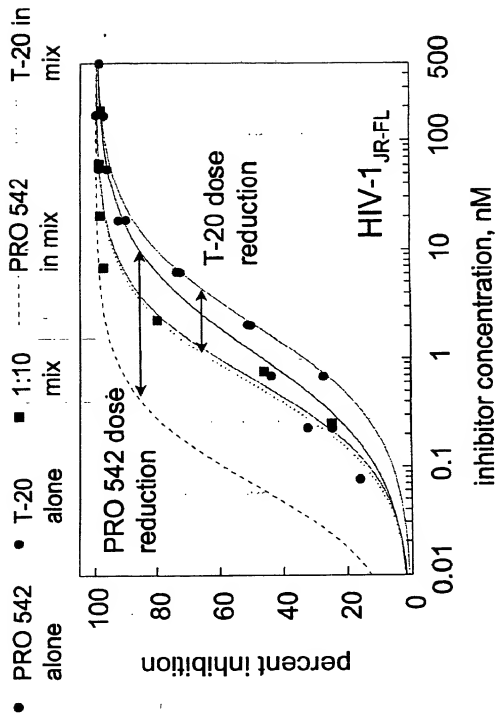


Figure 19

Synergistic Inhibition of Cell-Cell Fusion with PRO 542 and T-20 (I)

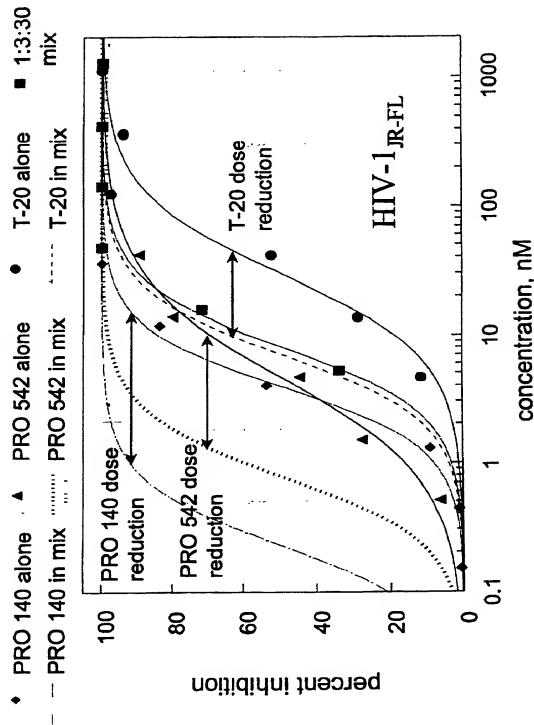


Synergistic Inhibition of HIV-1 Cell-Cell Fusion with PRO 542 and T-20 (II)

Conc. Ratio	Percent Inhibition	Combination Index	Inhibitory Conc, nM		Dose Reduction (fold)	
			PRO 542	T-20	PRO 542	T-20
1:2	95	0.32	95	47	17	4.9
	90	0.38	39	22	13	4.2
	50	0.69	3.0	2.5	6.2	2.8
1:10	95	0.27	28	58	20	4.8
	90	0.28	11	22	20	4.5
	50	0.34	0.84	1.3	22	3.7
1:50	95	0.33	47	120	56	3.2
	90	0.34	15	42	50	3.2
	50	0.38	0.49	1.8	35	3.0

Virus: HIV-1_{JR-FL}

PRO 140, PRO 542 and T-20 Triple Combination Synergistically Blocks HIV-1 Entry (I)



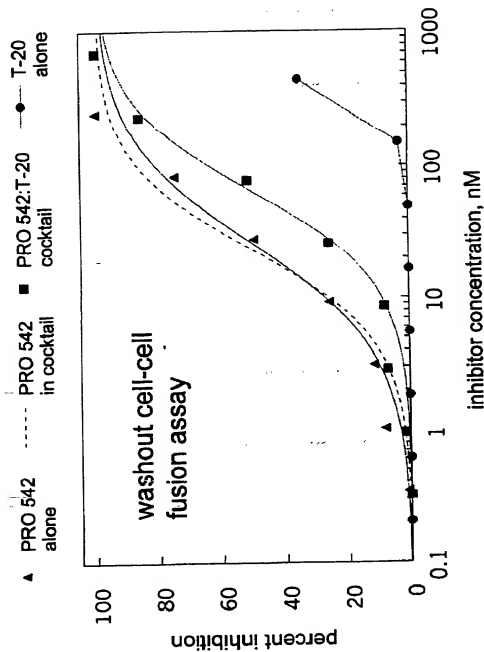
PRO 140, PRO 542, T-20 Triple Combination Synergistically Blocks HIV-1 Entry (II)

Percent Inhibition	Combination Index	Inhibitory Conc, nM			Dose Reduction (fold)		
		PRO 140	PRO 542	T-20	PRO 140	PRO 542	T-20
95	0.24	24	61	160	17	12	7.1
90	0.22	23	32	96	21	8.4	7.4
70	0.19	20	9.8	40	32	4.5	8.9
50	0.18	18	4.7	23	41	3.0	10

Inhibition of HIV-1_{JR-FL} mediated cell-cell fusion with PRO 140, PRO 542 and T-20 used in a 1:3:30 molar ratio.

Figure 23

PRO 542 Does Not Potentiate T-20 Activity in the Absence of Coreceptor



Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (I)

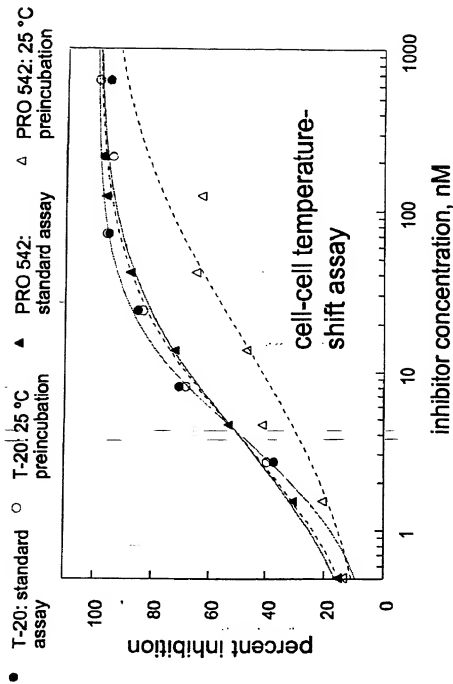


Figure 25

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (II)

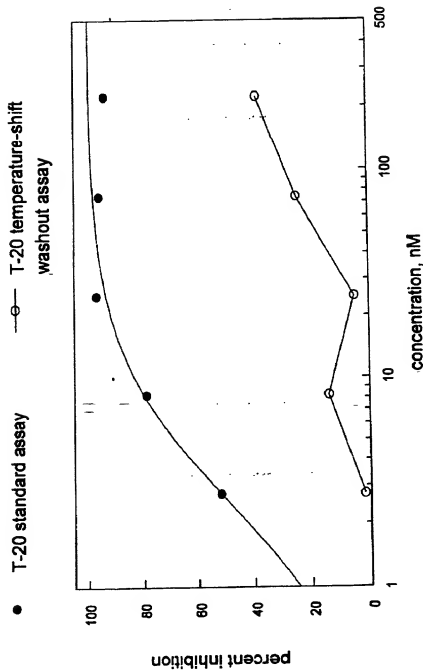
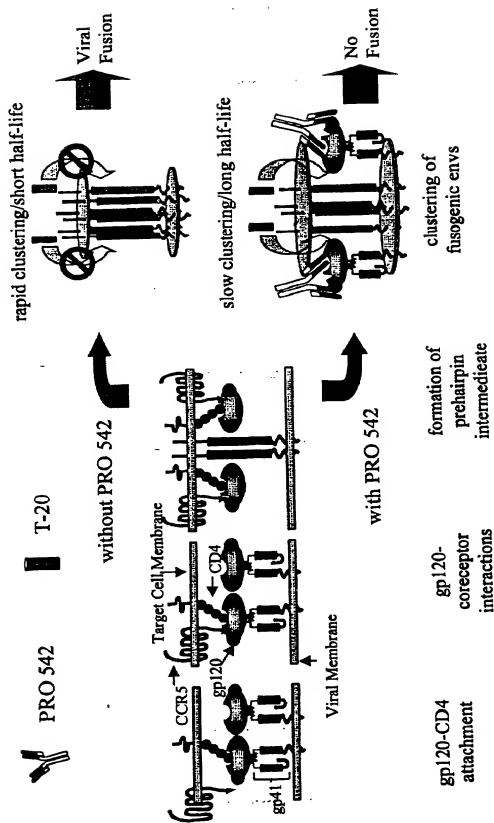


Figure 26

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets



Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

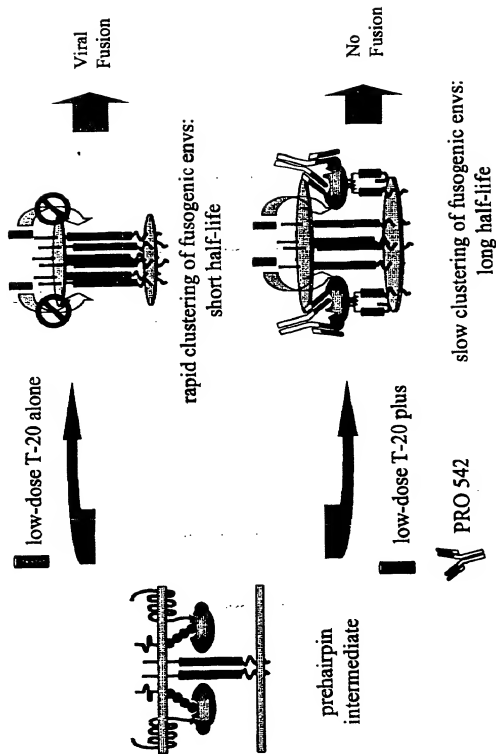


Figure 28

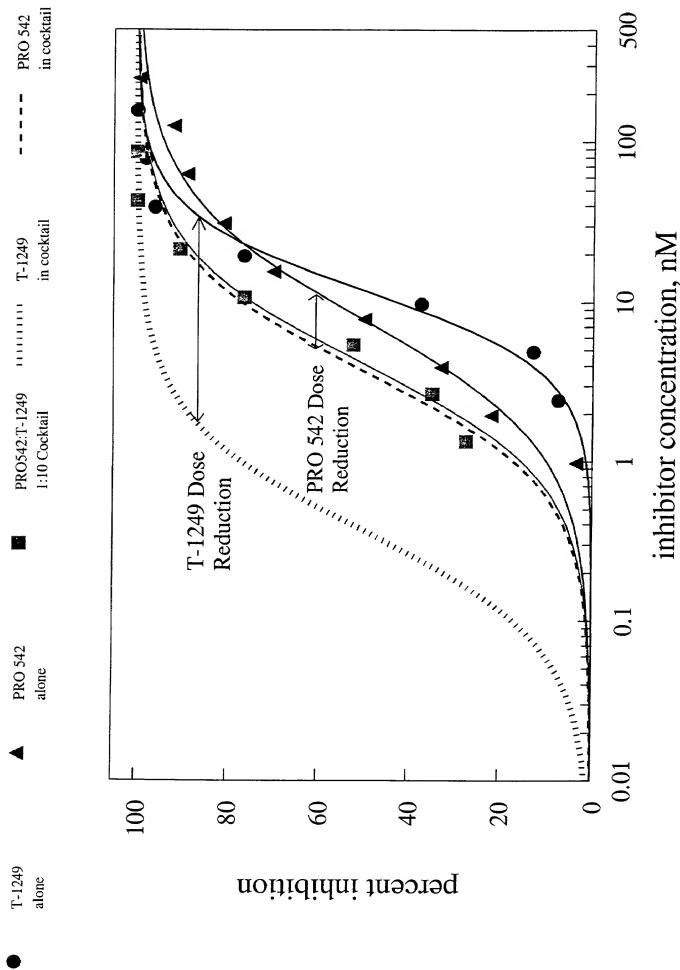


Figure 29

Fraction Inhibited	Dose PRO 542, nM (alone)	Dose PRO 542, nM (comb)	Dose T-1249, nM (alone)	Dose T-1249, nM (comb)	Combination Index	Dose PRO 542		Dose T-1249	
						Reduction	Index	Reduction	Index
0.95	87.90	13.58	37.83	1.36	0.20	6.47	0.20	27.86	0.20
0.90	48.69	9.52	27.11	0.95	0.24	5.12	0.24	28.48	0.24
0.85	33.78	7.64	22.06	0.76	0.27	4.42	0.27	28.87	0.27
0.80	25.65	6.47	18.88	0.65	0.30	3.96	0.30	29.17	0.30
0.75	20.43	5.65	16.61	0.56	0.32	3.62	0.32	29.42	0.32
0.70	16.75	5.01	14.85	0.50	0.34	3.34	0.34	29.64	0.34
0.65	13.99	4.50	13.41	0.45	0.37	3.11	0.37	29.84	0.37
0.60	11.81	4.06	12.20	0.41	0.39	2.91	0.39	30.03	0.39
0.55	10.05	3.68	11.13	0.37	0.41	2.73	0.41	30.21	0.41
0.50	8.57	3.35	10.18	0.33	0.44	2.56	0.44	30.39	0.44